GLOMALIN EXTRACTION

(Wright *et al*., 1996, Wright & Upadhyaya, 1996, & Wright & Upadhyaya, 1998)

INTRODUCTION

The following procedures may be used to remove glomalin from field soil, roots, mesh strips or bags, or pot culture media. This extract may then be used in further analyses (e.g. ELISA, Bradford, and dot blot assays). Either the total protein or easily extractable protein procedure may be used, depending on what is desired. The total protein extraction, gives a total protein concentration, which may have reduced immunoreactivity. Whereas the easily extractable protein extraction gives the most immunoreactive fraction but is not necessarily a measure of the total protein concentration in the sample.

Total Protein Extraction

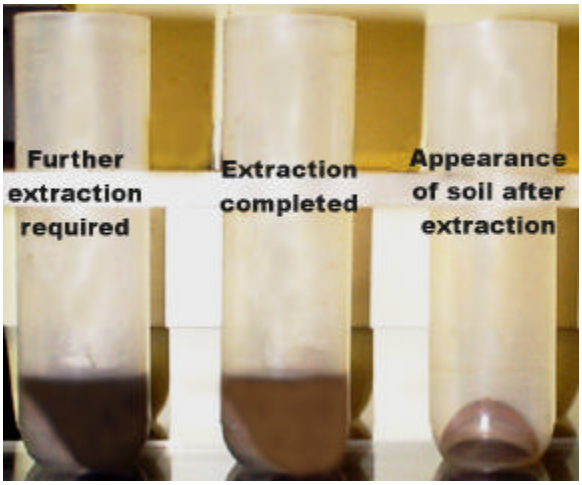
Materials:

autoclavable centrifuge tubes
50mM sodium citrate (citric acid, tri-sodium salt dihydrate), pH 8.0
graduated cylinder

Methods

1. Place 1.0 g of soil in a centrifuge tube with 8 ml 50mM sodium citrate (Smaller samples may be extracted as long as this ratio is adhered to).
2. Autoclave for 60-90 min at 121^{o}C (A 60 min extraction is usually performed.)
3. Centrifuge at 5000 xg for 15 min immediately after extraction (Centrifugation is just to pellet the soil particles and may be conducted at any speed from 3000-10000 xg).
4. Remove the supernatant containing the protein and store* at 4° C
5. Repeat steps 2-4 until you can easily see through the extract**
6. Measure total volume of extract with a graduated cylinder

This is an example of how a typical sample will look following extraction.



Easily Extractable Protein Extraction

Materials:

autoclavable centrifuge tubes
20mM sodium citrate (citric acid, tri-sodium salt dihydrate), pH 7.0
graduated cylinder

Methods

1. Place 1.0 g of soil in a centrifuge tube with 8 ml 20mM sodium citrate (Smaller samples may be extracted as long as this ratio is adhered to).
2. Autoclave for 30-60 min. at 121C (A 30 min extraction may be used on soil to remove the fresh protein and compare with the 60 min total extraction. However, the 60 min extraction is used for pot cultures as a total protein assay.)
3. Centrifuge at 5000 xg for 15 min immediately after extraction (Centrifugation is just to pellet the soil particles and may be conducted at any speed from 3000-10000 xg).
4. Remove the supernatant that contains the protein and store* at 4° C
5. Measure total volume of extract with a graduated cylinder

* Protein may be stored in this manner for 2-4 weeks while the other analyses are conducted. Samples must be observed for the growth of contaminants, at which point samples can no longer be used. If desired, 1-ml subsamples can be transferred from extraction tubes to microtiter tubes (recentrifuge at 10,000 rpm for 3 minutes with a microcentrifuge). These subsamples are easy to transport and perform protein and ELISA analyses from rather than working with extraction tubes. The microtiter tubes are sold in boxes containing 96 tubes which corresponds nicely with analysis plates and with a mutlichannel pipetter. The extract may also be transferred to eppendorf tubes instead of the microtiter tubes and treated in a similar manner, but these tubes do not work well with multichannel pipetters.